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Acute and Subchronic Toxicity of Pentachlorobenzene

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16. ABSTRACT

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ACUTE AND SUBCHRONIC TOXICITY OF PENTACHLOROBENZENE

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Oral LD_{50} values for pentachlorobenzene (QCB) in rats were 1125, 1080, and 940 mg/kg for adult males, adult females, and weanling females, respectively. The oral LD_{50} values in mice were 1175 mg/kg for males and 1370 mg/kg for females. Clinical signs of toxicity included tremors and narcosis. Dermal application of 2500 mg/kg did not produce clinical signs in rats.

In subchronic studies, weanling male rats were fed 0, 125, or 1000 ppm QCB for 100 days and weanling females fed 0, 125, 250, 500, or 1000 ppm for 180 days. No clinical signs of toxicity or effects on growth were observed in these rats throughout the exposures. QCB accumulated in adipose tissues at approximately 1.5-2.2 times the dietary concentrations. Porphyrin measurements were made only in females. Terminal values for urinary uro-and coproporphyrin and accumulation of liver porphyrins were not remarkably different in control and QCB-treated groups. In groups fed 1000 ppm, the WBC was increased and red blood cell indices were generally decreased compared to controls. The rats were pair-bred with untreated partners after 67 days of treatment. Fertility and fecundity were unaffected in either sex; however, suckling pups of QCB-treated mothers fed 250 ppm or more developed tremors and at 1000 ppm most died before weaning. Adrenal weights in males and kidney weights in both sexes were increased in adults fed 1000 ppm. In groups fed 250 ppm or more liver/body weight ratios were increased in both adults and in weanling offspring of QCB-treated dams. Hepatocellular enlargement was particularly evident in the 500 and 1000 ppm groups. In the

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kidneys of adult males, more numerous and larger foci of tubular atrophy and lymphocytic infiltration were seen at 1000 ppm than were seen in controls and dose-related increases in hyaline droplet formation occurred at 125 and 1000 ppm.

INTRODUCTION

The chlorinated benzenes are commercially important chemicals of current toxicological interest. Pentachlorobenzene (QCB) has limited industrial use but is found in trace quantities as an impurity in technical grades of the fungicides pentachloronitrobenzene (Kuchar *et al.*, 1969) and hexachlorobenzene (Villanueva *et al.*, 1974). QCB can also occur as a metabolic product of hexachlorobenzene (Mehendale *et al.*, 1975) and the insecticide lindane (Karapally *et al.*, 1973). Plimmer and Klingebiel (1976) demonstrated, experimentally, that QCB is a photoproduct when hexachlorobenzene is exposed to sunlight in the presence of organic sensitizers. They speculated on the possiblity of environmental hexachlorobenzene, a widespread pollutant, undergoing similar reactions in the presence of naturally occurring photosensitizers. Like a number of aromatic organo-chlorine compounds, QCB appears to be persistent and is degraded only slowly in soil (Beck and Hansen, 1974). Presumably, biospheric contamination by QCB is slight, although the chemical has been tentatively identified in human fat (Morita *et al.*, 1975).

Existing information on the toxicology of QCB is limited. No subchronic or LD_{50} studies were found in the literature; however, a few studies suggest that QCB might have significant toxicological activity. In rats given 250 mg/kg for 3 days, QCB produced an increase in liver weight and induced a variety of hepatic drug metabolizing enzymes (Ariyoshi *et al.*, 1975). In teratogenesis studies, QCB accumulated in the fetus (Villeneuve and Khera, 1975), reduced fetal weight, and increased the incidence of extra ribs and sternal defects (Khera and Villeneuve, 1975).

The present communication reports the acute toxicity of QCB in rats and mice and the subchronic toxicity in rats. These studies were undertaken to expand the toxicological data base of QCB. In view of lactational and porphyrogenic effects of hexachlorobenzene (De Matteis *et al.*, 1961; Grant *et al.*, 1977) preliminary investigation of reproduction and porphyrin excretion was incorporated into the subchronic study.

MATERIALS AND METHODS

QCB (purity 99.1%) was provided by the Olin Corporation, Stanford, CT. No other chlorinated compounds were detected by GC-EC analysis of the sample. Sherman strain rats were obtained from the U.S. Center for Disease Control, Atlanta,GA. Swiss-Webster mice were purchased from Hilltop Laboratory Animals, Inc., Scottdale, PA.

Acute Studies

The general procedure described by Gaines (1960) was followed for the acute LD_{50} studies. In the oral tests QCB was dissolved in peanut oil and a single dose administered by stomach tube to 10 animals per dosage level. Weanling animals were 27-35 days of age and adult animals 90-120 days of age. Details of various dosages and volumes are given in Table 1. For dermal application, QCB was dissolved in xylene and applied to the shaved back and shoulder area of rats. A single dosage level of 2500 mg/kg was used in the dermal tests. Subsequent to dosing, animals in the acute tests were observed for 2 weeks or longer until they appeared clinically normal.

TABLE 1. Acute Toxicity of Pentachlorobenzene

Species	Route	Sex	Age	LD_{50} mg/kg	19/20 C.L. of LD_{50} mg/kg	Dosage Range Tested mg/kg	Dose Volume ml/kg
Rat	Oral	Male	Adult	1125	1015-1247	750-1350	7.5
Rat	Oral	Female	Adult	1080	952-1226	750-1500	7.5
Rat	Oral	Female	Weanling[2]	940	864-1023	600-1200	5.0
Mouse	Oral	Male	Adult	1175	1035-1334	750-1500	15.0
Mouse	Oral	Female	Adult	1370	1263-1487	1050-1500	15.0
Rat	Dermal	Male	Adult	>2500	-	2500[1]	5.0
Rat	Dermal	Female	Adult	>2500	-	2500[1]	5.0

[1]Only one dosage level tested.

[2]Weanlings were 27-35 days of age.

Subchronic Studies

Groups of ten 4-5 week-old weanling female rats were fed Purina Laboratory Chow fortified with QCB at dietary concentrations of 0, 125, 250, 500 and 1000 ppm. Similarly, groups of males were fed 0, 125 and 1000 ppm. The rats were caged 2 per wire-bottom cage and provided their respective diets *ad libitum*. Periodic measurements of food consumption were made and body weights recorded weekly. After 67 days of treatment, both males and females were pair-bred with previously untreated partners. QCB diets were suspended on males for 16-64 hr to prevent exposing the untreated females, but treated females were maintained on their respective diets throughout breeding, gestation and lactation. The resultant pregnant rats were allowed to whelp and their litters observed through weaning (21 days). From each litter weaned, one male and one female pup were selected randomly on the day weaned, necropsied, and their livers weighed. Livers, kidneys and adrenals of one male and one female pup from each weaned litter of the females were examined microscopically.

Adult male rats were killed after birth of their progeny resulting in a dietary exposure of 100 days. Feeding of the females was extended to obtain total exposure of 180 days. The week before sacrifice, a 24 hr urine sample was collected from each female rat. All adult animals (10 per group) were killed by exsanguination under ether anesthesia after collecting blood from the abdominal aorta for hematologic studies. Organs and tissues were inspected grossly and the viscera examined under long-wave U.V. light. The brain, heart, lungs, liver, kidneys, spleen, adrenals and testes were weighed. These organs and the thymus, trachea, thyroid, parathyroid, esophagus, stomach, intestines, pancreas, urinary bladder, uterus, ovaries, pituitary, epididymides, prostate, seminal vesicles, coagulating gland and lymph nodes were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. A section of liver was collected from each female and frozen pending assay for porphyrins. Perirenal fat samples were collected from 4 rats of each group for QCB residue analysis.

Urine from the females was assayed for uro- and coproporphyrin (Goldstein *et al.*, 1974) and liver sections from the females assayed for total porphyrin (Abbritti and De Matteis, 1971). Fat residues were extracted with hexane and GC-EC analysis made on 2 columns for confirmation. The column packings were 4% SE-30/6% OV-210 and 1.5% OV-17/1.95% OV-210 coated on 80/100 Gas Chrom Q. The column flows were 70 cc/min of 5% methane in argon and the column oven temperature was 175°C. LD_{50} values were calculated by the method of Litchfield and Wilcoxon (1949). Organ weights, body weights, hematologic measurements and porphyrin values were compared by analysis of variance followed by Duncan's multiple range test (Steel and Torrie, 1960).

RESULTS

Acute Toxicity

LD_{50} determinations of QCB are summarized in Table 1. The oral LD_{50} values were similar in rats regardless of age or sex, the values being 1125, 1080, and 940 mg/kg for adult males, adult females, and weanling females, respectively. In mice, the oral LD_{50} values were 1175 mg/kg for males and 1370 mg/kg for females. Dermal application of 2500 mg/kg did not produce clinical signs of toxicity in male or female rats.

When QCB was given by the oral route the most characteristic sign of acute poisoning was tremor which was usually observed in mice about 24 hr after dosing and in rats about 48 hr after dosing. Decreased activity and hypersensitivity to touch were noted prior to onset of tremor. In rats surviving several days, reddish stains around the mouth, nose and eyes were frequently seen. Weakness and labored breathing were commonly noted and some animals appeared narcose before death. The urine of a number of rats was a deep yellow color. Milder signs of toxicity (primarily light tremor) were observed in both rats and mice at sublethal dosage levels. In mice, most deaths occurred 2-4 days after dosing, but survival time was longer in rats with death

occurring 5-12 days after dosing in adult animals. Gross inspection of rats that died suggested enlargement of kidneys, livers, and adrenals. In some rats the gastric mucosa was hyperemic. When the viscera of dead rats and mice were examined under long-wave U.V. light, a slight reddish fluorescence of the G.I. tract was usually seen.

Subchronic Studies

None of the rats receiving post-weaning dietary exposure to QCB died, and none exhibited clinical signs of toxicity. Food consumption and body weight gain were unaffected by treatment. Daily dosage of QCB throughout exposure is estimated for the various groups in Fig. 1. Maximum dosage occurred in the females fed the 1000 ppm dietary concentraction in which the dosage was 134 mg/kg/day during the first week of exposure but declined to 55mg/kg/day at 6 months.

Reproduction and Lactation

Results of reproduction studies are presented in Table 2. Fertility, as judged by the number and size of the litters, was not overtly affected in either males or females. When first weighed at 4 days of age, offspring of mothers fed QCB were smaller than offspring of the control group, and pre-weaning growth rates were decreased in pups of mothers fed the higher dietary concentrations of QCB. Tremors developed 4-14 days after birth in suckling pups of mothers fed 250 ppm or more. Significant mortality occurred in pups of mothers fed 1000 ppm QCB, the survival rate from day 4 to weaning being only 28%. By the time of weaning, tremors were no longers evident, but a number of pups in the 500 and 1000 ppm groups had wetting around the urinary opening suggesting perhaps urinary incontinence or general weakness. Pups of mothers fed 125 ppm QCB were not remarkably different from pups of the control group. Treatment of males with 1000 ppm QCB had no discernible effect on reproduction or survival of pups.

Hematology

Compared to the control groups, RBC and hematocrit were decreased in males fed 1000 ppm and hemoglobin was decreased in both sexes fed 1000 ppm (Table 3). The WBC was increased in both sexes fed 1000 ppm. Mean cell volume and differential white cell counts in QCB-treated rats were comparable to controls.

Porphyrins

When the liver and viscera were viewed under long wavelength ultraviolet light at necropsy, the red fluorescence characteristic of the accumulation of porphyrins was not observed in male or female rats treated subchronically with QCB. Only tissues from female rats were analyzed for porphyrins, since male rats have been shown to be less susceptible than females to the porphyrogenic effects of hexachlorobenzene (Grant *et al.*, 1974). The excretion of

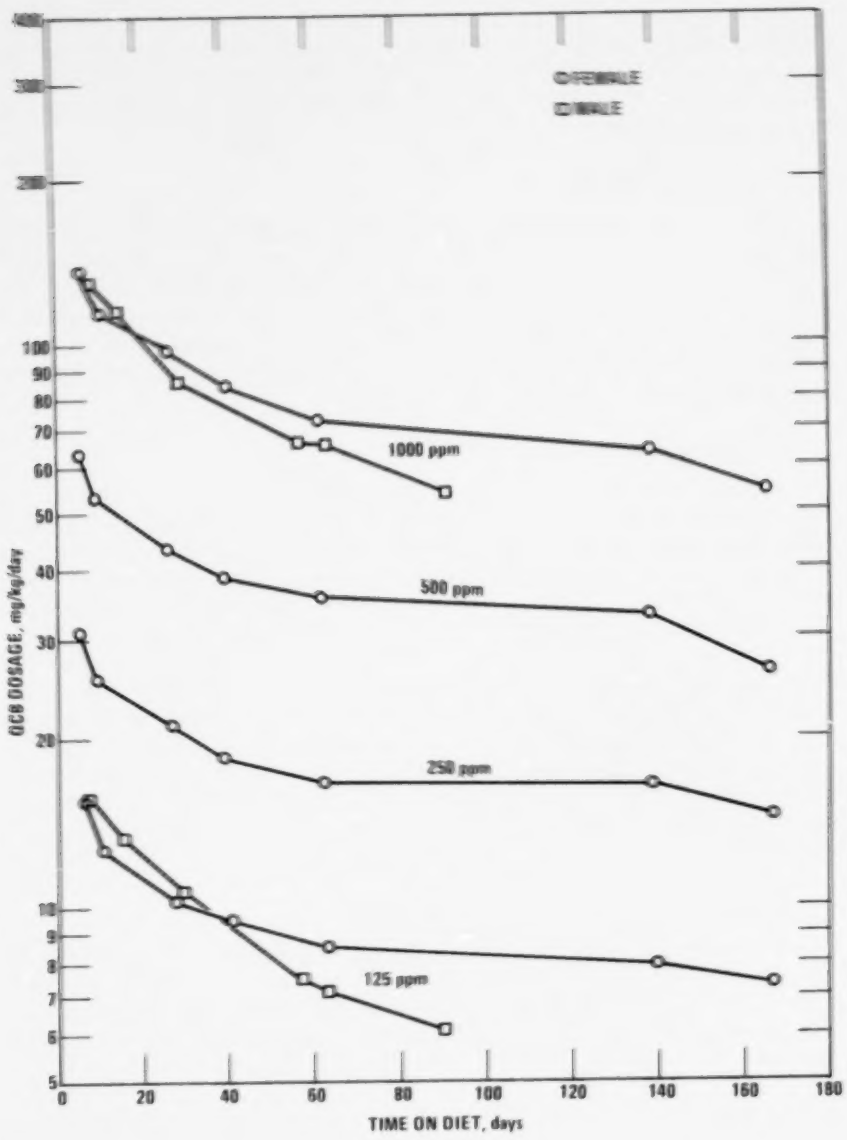

FIGURE 1. Estimated dietary dosage of QCB based on food consumption.

TABLE 2. Reproduction and Survival, Growth, and Liver Weights of Pups in Rats fed Pentachlorobenzene[1]

Diet Level ppm	Parent Treated	Litters Litters Born n	Pups/ Litter mean	Litters Weaned n	Survival Days 0-4 %	Survival Days 4-21 %	Pup Body Wts.[2] at Weaning Male g	Pup Body Wts.[2] at Weaning Female g	Liver Weight of Weanling Pups[3] Liver Weight Male g	Liver Weight of Weanling Pups[3] Liver Weight Female g	Liver/Body Wt. Ratio Male g/100g	Liver/Body Wt. Ratio Female g/100g
0	F	8	10.4	8	100.00	100.0	45±5	45±8	2.0±0.2	1.9±0.2	3.9±0.1	4.0±0.1
125	F	6	12.0	6	98.6	91.7	44±3	41±3	1.8±0.1	1.6±0.1	3.9±0.1	3.9±0.1
250	F	9	11.9	9	98.1	95.4	41±4	40±3	1.8±0.1	1.7±0.1	4.3±0.1[4]	4.2±0.1
500	F	9	13.2	9	98.3	68.6	40±4	38±4	2.2±0.1	2.1±.1	5.1±0.1[4]	5.3±.1[4]
1000	F	8	10.8	4	94.2	28.0	31±4	37±4	2.2±0.1	2.5±0.3[4]	6.5±0.1[4]	6.5±.2[4]
0	M	10	12.1	10	92.6	86.8	42±5	39±4	1.6±0.1	1.4±0.1	3.7±0.1	3.6±0.1
125	M	9	12.8	9	100.0	84.4	42±4	40±3	1.6±0.1	1.5±0.1	3.5±0.1	3.6±0.1
1000	M	10	12.6	10	86.5	87.9	39±4	37±3	1.4±0.1	1.4±0.1	3.6±.1	3.6±0.1

[1]Ten weanling male or female rats per group were fed the diets indicated and pair-bred with untreated partners after 67 days. Pups were culled to 10 per litter when 4 days of age.

[2]Litter taken as unit for calculations. Values are mean ± SD.

[3]Group means ± SEM for one male and one female pup from each surviving litter.

[4]Differs from control $p < 0.05$. Statistics done only on liver weights.

TABLE 3. Hematology and Porphyrin Excretion and Storage in Rats Fed Pentachlorobenzene[1]

Diet Level ppm	Sex	RBC	Hb	Ht	WBC	Urinary Excretion Coproporphyrin	Uroporphyrin	Liver Porphyrin
		10^6/mm^3	g/dl	%	10^3/mm^3	μg/24 hr	μg/24 hr	μg/g liver
0	F	7.66±0.09	14.93±0.07	39.61±0.69	5.75±0.36	3.19±0.49	1.20±0.03	0.64±0.03
125	F	7.53±0.12	14.92±0.15	38.38±0.60	5.68±0.68	2.98±0.30	1.14±0.07	0.66±0.02
250	F	7.47±0.08	14.71±0.14	38.31±0.68	5.86±0.21	4.65±0.61	1.22±0.09	0.72±0.04
500	F	7.47±0.13	14.57±0.24	37.88±0.61	5.50±0.41	5.40±1.10	1.20±0.06	0.70±0.02
1000	F	7.39±0.11	14.14±0.13[2]	37.15±0.71	7.63±0.44[2]	3.86±0.33	1.20±0.04	0.79±0.03[2]
0	M	7.89±0.17	14.84±0.21	44.60±0.84	8.11±0.40			
125	M	7.72±0.07	14.80±0.12	43.20±0.29	9.25±0.52			
1000	M	7.43±0.13[2]	14.21±0.14[2]	41.50±0.71[2]	10.25±0.57[2]			

[1]Weanling male or female rats were fed the diets indicated and pair-bred with untreated partners after 67 days. Values are group means ±SEM for males fed 100 days and females fed 6 months. Mean cell volume and differential leukocyte counts (not shown) were comparable to controls.

[2]Differs from control $p < 0.05$.

urinary uro- and coproporphyrins was not affected by QCB, even at 1000 ppm (Table 3). Total liver porphyrins were minimally higher in female rats fed 1000 ppm QCB than in controls (0.79 μg/g compared to 0.64 μg/g), but this slight difference was not judged to be a porphyrogenic response and is of doubtful significance.

Fat Residues

Results of residue analyses are presented in Table 4. QCB accumulation in adipose tissue was dose-dependent. Fat burdens of QCB were approximately 1.5-2.2 times the dietary concentration. Residues in males and females were not dissimilar, but cannot be compared directly because of the longer exposure period of the females and the complicating factors of pregnancy and lactation.

TABLE 4. Adipose Tissue Residues in Rats Fed Pentachlorobenzene[1]

Dietary Level ppm	Sex	Pentachlorobenzene in Fat ppm
0	F	<0.4-
125	F	184.0±24.3
250	F	431.0±40.2
500	F	1074.3±150.0
1000	F	2022.3±116.7
0	M	<0.4-
125	M	209.0±14.5
1000	M	1467.5±120.4

[1]Weanling male or female rats were fed the diets indicated and pair-bred with untreated partners after 67 days. Values are means ± SD of 4 rats per group for males fed 100 days and females fed 6 months.

Pathologic Findings

Tissues of 21 day-old weanlings appeared normal although relative weights (organ/body weight ratio) of the livers were increased in pups of mothers fed 250 ppm or more (Table 2). Light microscopic examination revealed no treatment related changes in the kidneys or adrenals of weanling offspring from QCB-treated mothers. The obvious change was hepatocellular enlargement in all pups of the groups fed 500 and 1000 ppm. Minimal enlargement of hepatic cells was observed in 2/9 male pups of the 250 ppm group. The cytoplasm of the enlarged cells was finely reticulated and clumps of basophilic material were scattered throughout. The nuclei of some of the cells were larger than normal. Some cells contained small or large vacuoles in the cytoplasm.

At necropsy of the adult rats, no remarkable changes were observed in tissues of males fed QCB for 100 days or females fed QCB for 180 days. Liver, kidney and adrenal weights of adult animals are given in Table 5. Compared to

TABLE 5. Organ Weights and Final Body Weights of Rats Fed Pentachlorobenzene[1]

Diet Level ppm	Sex	Final Weight g	Organ Weight Liver g	Organ Weight Kidneys g	Organ Weight Adrenals mg	Organ/Body Weight Ratio Liver g/100g	Organ/Body Weight Ratio Kidneys g/100g	Organ/Body Weight Ratio Adrenals mg/100g
0	F	286±5	8.80±0.18	1.70±0.03	48.32±1.12	3.08±0.05	0.60±0.01	16.80±0.40
125	F	291±5	9.08±0.26	1.78±0.04	47.87±2.46	3.13±0.06	0.61±0.01	16.50±0.90
250	F	288±7	9.57±0.26	1.80±0.05	50.24±1.94	3.33±0.05[2]	0.63±0.01	17.10±0.40
500	F	287±6	10.04±0.31[2]	1.81±0.04	48.90±2.18	3.50±0.08[2]	0.63±0.01	17.00±0.70
1000	F	286±6	11.88±0.47[2]	2.02±0.09[2]	53.88±3.87	4.15±0.12[2]	0.71±0.02[2]	19.00±1.00
0	M	427±11	12.53±0.53	2.51±0.08	38.12±1.23	2.93±0.06	0.60±0.01	9.02±0.44
125	M	460±16	13.62±0.59	2.82±0.12[2]	38.44±1.72	2.96±0.04	0.61±0.01	8.47±0.53
1000	M	403±10	17.39±0.71[2]	3.14±0.11[2]	44.82±2.71[2]	4.30±0.08[2]	0.78±0.03[2]	11.12±0.56[2]

[1]Weanling male or female rats were fed the diets indicated and pair-bred with untreated partners after 67 days. Values are group means ± SEM for males fed 100 days and females fed 180 days.
[2]Differs from controls $p < 0.05$.

the control groups, both absolute and relative liver weights were increased in groups fed 500 or 1000 ppm, but only relative liver weights were increased in females fed 250 ppm. In groups fed 1000 ppm, absolute and relative adrenal weights in males and kidney weights in both sexes were increased. Absolute kidney weights were increased in males fed 125 ppm, but the relative weights did not differ from the controls. Statistically significant differences ($P < 0.05$) noted in other organs included increased relative weights of the brain and lungs in males fed 1000 ppm and decreased relative heart weights in males fed 125 ppm. Spleen and testis weights of QCB-treated rats were comparable to controls.

Microscopically, the significant change in female rats was hypertrophy of hepatic cells in the centrilobular regions of the liver. The cytoplasm of the enlarged cells tended to be finely reticulated with the basophilic component appearing as prominent clumps scattered throughout the cell. Occasionally the cytoplasm was finely vacuolated. The enlargement was evidenced by an increase in the cytoplasm, but often nuclei were also increased in size. The liver change was observed in all female rats fed 500 or 1000 ppm QCB, but was much less obvious in the 500 ppm group. In females fed 250 ppm, 2/10 showed equivocal minimal enlargement of hepatic cells. Liver changes in males fed 1000 ppm were similar to those described in females. Enlargement of hepatic cells was not observed in either sex fed 125 ppm.

No significant changes were observed in the kidneys of females except in one animal fed 1000 ppm. In this rat many of the renal tubules were dilated and filled with abundant eosinophilic proteinaceous material and the cytoplasm of some epithelial cells contained hyaline droplets. In contrast to the females, a dose-related increase in hyaline droplet formation was observed in the kidneys of male rats. In 9 control males a few small focally distributed hyaline droplets were seen in the cytoplasm of occasional epithelial cells of the proximal convoluted tubules. These droplets were apparent in all males fed 125 ppm QCB, but in 8 rats they were more prominent than in controls and many tubules throughout the kidney were involved. Similar changes in the 1000 ppm group were even more extensive and prominent. Another finding in males was the presence of foci in the renal cortex consisting of atrophic tubules and interstitial lymphocytic infiltration. These foci were observed in 2 controls and one rat fed 125 ppm, but were present to a greater degree, being more numerous and usually larger, in all males fed 1000 ppm and, in some rats, were associated with dilated tubules containing hyaline or granular casts.

In other organs of both control and treated rats, various minimal changes were observed that were not considered related to treatment. These included perivascular infiltration of lymphocytes in the lungs and slight to moderate hemosiderosis in the spleen, focal hemosiderosis in lymph nodes, and minimal to slight chronic inflammation in the trachea. In females, a common finding was the presence of dilated sinusoids in the adrenal glands.

DISCUSSION

On the basis of the LD_{50} values (Table 1), QCB can be considered moderately toxic. Gross evidence of liver and kidney effects was noted in rats dying from a

single dose and tremors occurred at dosages well below the LD_{50}. Compared with other chlorinated benzenes, QCB appears to have greater acute toxicity in the rat than monochlorobenzene (2.9 g/kg) and dichlorobenzenes (1-4 g/kg), but is roughly similar in toxicity to 1,2,4-trichlorobenzene (756 mg/kg) and 1,2,4,5-tetrachlorobenzene (1500 mg/kg) (Irish, 1963; Brown *et al.*, 1969; Fomenko, 1965). The dermal dosage of 2500 mg/kg used in the present study approached the maximum dosage that could be applied under the test conditions. Absence of clinical effects suggested that percutaneous absorption of QCB is rather poor.

Although clinical signs were not observed in the parents, suckling pups whose mothers were fed 250 ppm QCB or more developed tremors and at 1000 ppm most died before weaning. This result is presumptive evidence for excretion of a toxic agent via the milk. Suckling pups also appear more sensitive than their mothers to the related compound, hexachlorobenzene (Grant *et al.*, 1977). In lacteal exposure, however, it is difficult to clearly separate effects related to sensitivity from those related to dosage. Since QCB accumulates in the fetus Villaneuve and Khera, 1975), prenatal exposure of pups may also have contributed to effects observed in the present study.

The hepatic and renal effects associated with subchronic exposure to QCB suggest some degree of cumulative toxicity. Significant liver changes (increased weight and hepatocellular enlargement) occurred at the 500 and 1000 ppm dietary concentractions. In the kidneys of male rats fed 1000 ppm, focal areas of tubular atrophy and interstitial lymphocytic infiltration were more frequent and larger than in controls. Increased hyaline droplet formation in the proximal tubules was evident at both 125 and 1000 ppm. The droplets are generally believed to represent excess protein that has passed through the glomeruli and resorbed into the tubular epithelium and thus are an indication of proteinuria. Therefore, the glomeruli in treated males may have been more permeable than in the controls. Predisposition of males to this condition is consistent with reports indicating greater protein excretion in the male rat (Gray, 1977).

The toxicity of QCB is consistent with that of other chlorinated benzenes, the liver, kidneys and nervous system being the primary target organs. For example, *o*-dichlorobenzene was shown to affect the liver, kidneys and CNS (Hollingsworth *et al.*, 1958). Effects from various exposures to *p*-dichlorobenzene included tremors, increased liver and kidney weight, and cast formation in kidney tissue (Hollingsworth *et al.*, 1956). Chronic effects were seen only with relatively high dosages. Similarly, 1,2,4-trichlorobenzene produced hepatocytomegaly and hyaline degeneration in the kidney cortex (Coate *et al.*, 1977). High dietary dosages of hexachlorobenzene were associated with tremors and increased liver and kidney weight (Kimbrough and Linder, 1974).

No evidence of porphyria was observed in female rats exposed subchronically to QCB (Table 3). By comparison, hexachlorobenzene, a well known porphyrogenic chemical (De Matteis *et al.*, 1961), produces a massive accumulation of porphyrins in the liver and increased urinary excretion of these compounds after a lag period of several weeks. Cutaneous lesions and a red fluorescence of the liver are observed. Dietary administration of hexachloro-

benzene to female rats for 4 months at levels of 100 ppm (Goldstein *et al.*, 1978) and 8 mg/kg/day (Kuiper-Goodman *et al.*, 1977) results in 100-fold or more increases in liver porphyrins and urinary excretion of uroporphyrin. Although grossly toxic levels of QCB were not achieved in the present study, female rats fed 1000 ppm (134-55 mg/kg/day) for 6 months did not develop porphyria at approximately 10 times the porphyrogenic level reported for hexachlorobenzene.

Similarly, Carlson (1977) has shown that 50-200 mg/kg/day of hexachlorobenzene produces porphyria in female rats after 1-4 months. Hepatic and urinary porphyrins were elevated more than 100-fold, but 1,4-dichloro- and 1,2,4-trichlorobenzene were not porphyrogenic under identical conditions. These results contrast with earlier results of Rimington and Ziegler (1963) who reported that mono-, di-, tri- and tetrachlorobenzenes produce porphyria. However, these authors gave massive doses approaching the lethal dosage. Poland *et al.* (1971) found the porphyrinuria produced by similar doses of dichlorobenzenes to be rather minimal and transient. In the present study, we noted a slight red fluorescence characteristic of porphyrins in the G.I. tract of rats and mice dying from a single dose of QCB; however, subchronic administration of QCB did not produce any appreciable increase in the urinary excretion or hepatic accumulation of porphyrins indicating that it is weak or inactive as a porphyrogenic chemical.

The adipose levels of QCB suggest bioaccumulative properties for this compound. In male rats fed 1000 ppm QCB, deposition in the fat was 1467 ppm or 1.5 times the dietary concentration. With similar duration of exposure, accumulation of hexachlorobenzene is much greater (Kuiper-Goodman *et al.*, 1977). Male rats fed 213-580 ppm hexachlorobenzene (32 mg/kg/day) had fat burdens of 3902 ppm, approximately 7-18 times the dietary concentrations. In females, accumulation was even greater and marked cumulative toxicity was observed. The present study indicates that QCB shares several of the toxicological properties of other chlorinated benzenes; however, the chronic hazard associated with bioaccumulation and cumulative toxicity is considerable less than hexachlorobenzene.

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